PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A61K 39/385

A1

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(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

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(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: VACCINES AGAINST *ESCHERICHIA COLI* O157 INFECTION

(57) Abstract

This invention relates to conjugates of the O–specific polysaccharide of *E. coli* O157 with a carrier, and compositions thereof, and to methods of using of these conjugates and/or compositions thereof for eliciting an immunogenic response in mammals, including responses which provide protection against, or reduce the severity of, bacterial infections. More particularly it relates to the use of polysaccharides containing the tetrasaccharide repeat unit: (→3)–α–*D*–Gal*p*NAc–(1→2)–α–*D*–Per*p*NAc–(1→3)–α–*L*–Fuc*p*–(1→4)–β–*D*–Glc*p*–(1→, and conjugates thereof, to induce serum antibodies having bactericidal (killing) activity against hemolytic–uremic syndrome (HUS) causing *E. coli*, in particular *E. coli* O157. The conjugates, and compositions thereof, are useful as vaccines to induce serum antibodies which have bactericidal or bacteriostatic activity against *E. coli*, in particular *E. coli* O157, and are useful to prevent and/or treat illnesses caused by *E. coli* O157. The invention further relates to the antibodies which immunoreact with the O–specific polysaccharide of *E. coli* O157 and/or the carrier, that are induced by these conjugates and/or compositions thereof. The invention also relates to methods and kits using one or more of the polysaccharides, conjugates or antibodies described above.

FOR THE PURPOSES OF INFORMATION ONLY

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 424/193.1, 196.11, 197.11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/193.1, 196.11, 197.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: E.coli, protein, polysaccharide, vaccines

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,773,007 A (PENNEY et al) 30 June 1998, see col. 3-4 and claims 1-22, see entire document.	10-11
Y	US 4,711,779 A (PORRO et al) 08 December 1987, see abstract and claims 1-2, see entire document.	10-11
Y	US 4,356,170 A (JENNINGS et al) 26 October 1982, see abstract, claims 1-3 and entire document.	10-11
Y	US 5,693,326 A (LEES) 02 December 1997, see abstract, figures, columns 7-12, claims 1-19 and entire document.	1-21, 30-39

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 SEPTEMBER 1998	Date of mailing of the international search report 20 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GINNY PORTNER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	KONADU, E.Y. et al, Investigational Vaccine for Escherichia coli O157: Phase 1 study of O157 O-specific polysaccharide-Pseudomonas aeruginosa Recombinant Exoprotein A conjugates in Adults. The Journal of Infectious Diseases. February 1998, Vol. 177, pages 383-387, see page 386, column 2 and entire document.	1, 10-17, 19-26, 34,30-34 40 ---------- 2-9,27-29, 18,35-39, 41
Y	CRYZ, S.J. et al. Synthesis and Characterization of Escherichia coli O18 O-polysaccharide conjugate vaccines. Infection and Immunity. February 1990, Vol. 58, No. 2, pages 373-377, see entire document.	2,36
Y	TAYLOR, D.N. et al, Synthesis, characterization, and clinicla evaluation of Conjugate vaccines composed of the O-specific polysaccharide of Shigella dysenteriae Type 1, Shigella flexneri Type 2a, and Shigella sonnei (Plesiomonas shigelloides) bound to Bacterial toxoids. Infection and Immunity. September 1993, Vol. 61, No. 9, pages 3678-3687, see abstract and entire document.	34-36, 39
Y	SJOGREN,R. et al. Influence of Shiga-like toxin production in enteric infection with an enteropathogenic Escherichia coli strain. Gastroenterology. May 1987, Vol. 92, No. 5 part 2, page 1643, column 1, second abstract. see entire document.	34-39
Y	ROBBINS, J.B. et al. O-specific side chain toxin-protein conjugates as Parenteral vaccines for the prevention of Shigellosis and related Diseases. Reviews of Infectious Diseases. 1991, Vol. 13, No. 4 supplement, pages S362-S365. see abstract, page S364 and entire document.	1, 10, 34
X -- Y	CHU, C. et al. Preparation, characterization, and immunogenicity of conjugates composed of the O-specific polysaccharide of Shigella dysenteriae Type I (Shiga's Bacillus) bound to Tetanus Toxoid. Infection and Immunity. December 1991. Vol. 59, No. 12, pages 4450-4458, see entire document.	40 ----- 34,39
Y	GUPTA, R.K. et al. Comparative immunogenicity of conjugates composed of Escherichia coli O111 O-specific polysaccharide, prepared by treatment with Acetic acid or Hydrazine, bound to tetanus toxoid by two synthetic schemes. Infection and Immunity. August 1995, Vol. 63, No. 8, pages 2805-2810, see entire document.	34-35,39
X	KONADU, E.et al. Preparation, characterization, and immunological properties in mice of Escherichia coli O157 O-specific polysaccharide-protein conjugate vaccines. Infection and Immunity. November 1994, Vol. 62, No. 11, pages 5048-5054, see entire document.	10-22, 24-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,E	US 5,785,973 A (BIXLER et al) 28 July 1998, see claims 1-2 and entire document.	10-11, 13-17
Y	US 5,585,100 A (MOND et al) 17 December 1996, abstract, claims, chart 1, column 11, see entire document.	10-11, 13-17
Y	US 5,371,197 A (MARBURG et al) 06 December 1994, see column 6, line 51, column 7, line 10, see entire document.	10-18
A	DICK, W.E. Jr. et al. Glycoconjugates of Bacterial carbohydrate antigens, a survey and consideration of design and preparation factors. Conjugate Vaccines. 1989, Vol. 10, pages 48-114, see entire document.	1-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/385

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)S 1-9, 19-21 and 30-33, drawn to E.coli O157 O-specific polysaccharide conjugates which are covalently bound to one of four(4) different protein carriers, wherein the carrier is derived from Shiga toxin 1 or 2.
Group II, claim(s)10-18, drawn to E.coli O157 polysaccharide covalently bound to any protein, wherein various at least 6 species of protein carriers are recited.
Group III, claim(s) 22-26, drawn to antibodies which are immunoreactive with E. coli O157 O-specific polysaccharide.
Group IV, claim(s)27-29, drawn to a method of passively immunizing a host against O157 infection.
Group V, claim(s) 34-39, drawn to conjugates comprising O-specific polysaccharide from E.coli or Shigella dysentariae,(at least 4 different sources are recited) together with any one of four different protein carriers.
Group VI, claim(s)40, drawn to antibodies which are immunoreactive with Shiga toxin 1 or 2.
Group VII, claim(s) 41, drawn to a method of administering antibodies to a mammal.
This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:
GROUP I:(1) O157-BETA SUBUNIT OF SHIGA TOXIN 1, (2) O157-BETA SUBUNIT OF SHIGA TOXIN 2, (3)O157-NON-TOXIC MUTANT SHIGA TOXIN 1, HOLOTOXIN, (4)O157-NON-TOXIC MUTANT SHIGA TOXIN 2, HOLOTOXIN. GROUP II: (1)O157-TOXOID CONJUGATE, (2) O157-CLOSTRIDIUM TOXOID OR EXOTOXIN, (3)O157-PSEUDOMONAS AERUGINOSA RECOMBINANT EXOPROTEIN A, (4)O157-HEPATITIS B SURFACE ANTIGEN, (5)O157-HEPATITIS B CORE ANTIGEN, (6)O157-BOVINE SERUM ALBUMIN. GROUP V:(1)O111-SHIGA TOXIN, (2)O17-SHIGA TOXIN, (3)O26-SHIGA TOXIN, (4) SHIGELLA DYSENTERIAE O-SPECIFIC POLYSACCHARIDE-SHIGA TOXIN.

The inventions listed as Groups I,II,III,IV,V,VI,and VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the inventions differ in the structural components used in the invention and therefore differ in the function and effect derived from each, as well as the special technical feature set forth in Group II is known in the art, specifically O-specific polysaccharide-protein conjugates of Escherichia coli O157 to bovine serum albumin, Clostridium welchii exotoxin and Pseudomonas aeruginosa recombinant exoprotein A and therefore does not define an advancement in the art; therefore a special technical feature is not set forth therein.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the species contained in the different Groups comprise structural proteins or O-specific polysaccharide which are associated with differing diseases and contain different types of amino acids or sugars which in turn define differing structural components which work to produce different functions and effects. Therefore, each specifies defines a different invention.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: WILLIAMS S. FEILER
MORGAN AND FINNEGAN, L.L.P.
345 PARK AVENUE
NEW YORK, NEW YORK 10154

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 2026-4282PC	Date of Mailing *(day/month/year)*
International application No. PCT/US98/14976	**FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES	International filing date *(day/month/year)* 20 JULY 1998

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer GINNY PORTNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/220 (January 1994)☆

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: WILLIAMS S. FEILER
MORGAN AND FINNEGAN, L.L.P.
345 PARK AVENUE
NEW YORK, NEW YORK 10154

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 2026-4282PC	Date of Mailing *(day/month/year)* 20 OCT 1998 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US98/14976	International filing date *(day/month/year)* 20 JULY 1998

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

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[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

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4. **Further action(s):** The applicant is reminded of the following:

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer GINNY PORTNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/220 (January 1994)☆

(See notes on accompanying sheet)

CASE 2026-4282PC ATTY MJM
DUE 12/20/98
1 MO. CALL-UP 11/20/98
Reply Sch Rpt BY ES

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

CASE 2026-4282PC ATTY MJM
DUE 1/20/99
1 MO. CALL-UP 12/20/98
U.S. Suppl IDS BY ES

Applicant's or agent's file reference 2026-4282PC	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US98/14976	International filing date *(day/month/year)* 20 JULY 1998	(Earliest) Priority Date *(day/month/year)* NONE

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report. ✓

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [X] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**, ✓
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. ____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/14976

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 39/385

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)S 1-9, 19-21 and 30-33, drawn to E.coli O157 O-specific polysaccharide conjugates which are covalently bound to one of four(4) different protein carriers, wherein the carrier is derived from Shiga toxin 1 or 2.
Group II, claim(s)10-18, drawn to E.coli O157 polysaccharide covalently bound to any protein, wherein various at least 6 species of protein carriers are recited.
Group III, claim(s) 22-26, drawn to antibodies which are immunoreactive with E. coli O157 O-specific polysaccharide.
Group IV, claim(s)27-29, drawn to a method of passively immunizing a host against O157 infection.
Group V, claim(s) 34-39, drawn to conjugates comprising O-specific polysaccharide from E.coli or Shigella dysentariae,(at least 4 different sources are recited) together with any one of four different protein carriers.
Group VI, claim(s)40, drawn to antibodies which are immunoreactive with Shiga toxin 1 or 2.
Group VII, claim(s) 41, drawn to a method of administering antibodies to a mammal.
This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

GROUP I:(1) O157-BETA SUBUNIT OF SHIGA TOXIN 1, (2) O157-BETA SUBUNIT OF SHIGA TOXIN 2, (3)O157-NON-TOXIC MUTANT SHIGA TOXIN 1, HOLOTOXIN, (4)O157-NON-TOXIC MUTANT SHIGA TOXIN 2, HOLOTOXIN. GROUP II: (1)O157-TOXOID CONJUGATE, (2) O157-CLOSTRIDIUM TOXOID OR EXOTOXIN, (3)O157-PSEUDOMONAS AERUGINOSA RECOMBINANT EXOPROTEIN A, (4)O157-HEPATITIS B SURFACE ANTIGEN, (5)O157-HEPATITIS B CORE ANTIGEN, (6)O157-BOVINE SERUM ALBUMIN. GROUP V:(1)O111-SHIGA TOXIN, (2)O17-SHIGA TOXIN, (3)O26-SHIGA TOXIN, (4) SHIGELLA DYSENTERIAE O-SPECIFIC POLYSACCHARIDE-SHIGA TOXIN.

The inventions listed as Groups I,II,III,IV,V,VI,and VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the inventions differ in the structural components used in the invention and therefore differ in the function and effect derived from each, as well as the special technical feature set forth in Group II is known in the art, specifically O-specific polysaccharide-protein conjugates of Escherichia coli O157 to bovine serum albumin, Clostridium welchii exotoxin and Pseudomonas aeruginosa recombinant exoprotein A and therefore does not define an advancement in the art; therefore a special technical feature is not set forth therein.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the species contained in the different Groups comprise structural proteins or O-specific polysaccharide which are associated with differing diseases and contain different types of amino acids or sugars which in turn define differing structural components which work to produce different functions and effects. Therefore, each specifies defines a different invention.

15

PATENT COOPERATION TREATY

PCT

REC'D 01 DEC 2000

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
2026-4282PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/14976	20 JULY 1998	NONE

International Patent Classification (IPC) or national classification and IPC
IPC(7): A61K 39/385 and US Cl.: 424/193.1, 196.11, 197.11

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [X] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04 FEBRUARY 2000	08 NOVEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Joyce Bridgers for GINNY PORTNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/14976

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-32, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the claims:
pages 33-37, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [X] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/14976

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

Please See Supplemental Sheet.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/14976

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	2-5,7,9,27-29,35-39,41	YES
	Claims	1,6,8,10-26,30-34,40	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-41	NO
Industrial Applicability (IA)	Claims	1-41	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1,6,8, 10-17, 19-26, 30-34 and 40 lack novelty under PCT Article 33(2) as being anticipated by Konadu et al (1998).

Konadu et al disclose Escherichia coli O157:H7, O specific polysaccharide-B subunit of Shiga toxin 1(see page 386, col. 2, first paragraph). The antibodies induced were neutralizing antibodies directed against the Shiga toxin. The reference suggests that evaluation of other Shiga toxin toxoid proteins as carriers. Administration of the disclosed composition to mice would be in a pharmaceutical carrier and therefore inherently comprises a pharmaceutical carrier with the polysaccharide-protein conjugate composition. The use of recombinant Pseudomonas aeruginosa exoprotein A as a carrier protein is also disclose (title of article). The dose for the administered polysaccharide is disclosed to be 25 ug of the E.coli O157:H7 polysaccharide (page 384, col. 1, paragraph 1). A method of inducing an immune response using the disclosed vaccine composition is disclosed to have induced antibodies to both the polysaccharide and the shiga toxin carrier protein. Clinical trials in humans were shown to provide encouraging test results, wherein one human subject upon infection with E.coli O157:H7 after having been vaccinated with the conjugate composition evidenced a positive stool culture for Ecoli O157:H7 but not adverse reaction and a negative stool culture at repeat testing (page 384, col. 1, clinical response). Serum samples obtained from patients evidenced immunoreactivity against Shiga toxin 1 beta subunit and therefore anticipates the claimed antibody compositions of claim 40.

Claims 10-22 and 24-26 lack novelty under PCT Article 33(2) as being anticipated by Konadu et al (1994).

Konadu et al disclose the production of Escherichia coli O157:H7 polysaccharide-protein conjugates for use as vaccines, wherein the conjugates are produced with a hydrazine linker or through acetic acid hydrolysis. Antibodies specific to both the polysaccharide and the protein carrier where identified in serum samples taken after vaccination of the host. Therefore, the

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

I. BASIS OF REPORT:

5. (Some) amendments are considered to go beyond the disclosure as filed:
NONE

IV. LACK OF UNITY OF INVENTION:

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2, and 13.3 is not complied with for the following reasons:
This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)S 1-9, 19-21 and 30-33, drawn to E.coli O157 O-specific polysaccharide conjugates which are covalently bound to one of four(4) different protein carriers, wherein the carrier is derived from Shiga toxin 1 or 2.
Group II, claim(s)10-18, drawn to E.coli O157 polysaccharide covalently bound to any protein, wherein various at least 6 species of protein carriers are recited.
Group III, claim(s) 22-26, drawn to antibodies which are immunoreactive with E. coli O157 O-specific polysaccharide.
Group IV, claim(s)27-29, drawn to a method of passively immunizing a host against O157 infection.
Group V, claim(s) 34-39, drawn to conjugates comprising O-specific polysaccharide from E.coli or Shigella dysentariae,(at least 4 different sources are recited) together with any one of four different protein carriers.
Group VI, claim(s)40, drawn to antibodies which are immunoreactive with Shiga toxin 1 or 2.
Group VII, claim(s) 41, drawn to a method of administering antibodies to a mammal.
This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:
GROUP I:(1) O157-BETA SUBUNIT OF SHIGA TOXIN 1, (2) O157-BETA SUBUNIT OF SHIGA TOXIN 2, (3)O157-NON-TOXIC MUTANT SHIGA TOXIN 1, HOLOTOXIN, (4)O157-NON-TOXIC MUTANT SHIGA TOXIN 2, HOLOTOXIN. GROUP II: (1)O157-TOXOID CONJUGATE, (2) O157-CLOSTRIDIUM TOXOID OR EXOTOXIN, (3)O157-PSEUDOMONAS AERUGINOSA RECOMBINANT EXOPROTEIN A, (4)O157-HEPATITIS B SURFACE ANTIGEN, (5)O157-HEPATITIS B CORE ANTIGEN, (6)O157-BOVINE SERUM ALBUMIN. GROUP V:(1)O111-SHIGA TOXIN, (2)O17-SHIGA TOXIN, (3)O26-SHIGA TOXIN, (4) SHIGELLA DYSENTERIAE O-SPECIFIC POLYSACCHARIDE-SHIGA TOXIN.

The inventions listed as Groups I,II,III,IV,V,VI,and VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the inventions differ in the structural components used in the invention and therefore differ in the function and effect derived from each, as well as the special technical feature set forth in Group II is known in the art, specifically O-specific polysaccharide-protein conjugates of Escherichia coli O157 to bovine serum albumin, Clostridium welchii exotoxin and Pseudomonas aeruginosa recombinant exoprotein A and therefore does not define an advancement in the art; therefore a special technical feature is not set forth therein.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the species contained in the different Groups comprise structural proteins or O-specific polysaccharide which are associated with differing diseases and contain different types of amino acids or sugars which in turn define differing structural components which work to produce different functions and effects. Therefore, each specifies defines a different invention.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
reference anticipates the now claimed invention.

Claim 40 lacks novelty under PCT Article 33(2) as being anticipated by Chu et al(1991).

Chu et al disclose a composition of antibodies produced through the immunization of a host animal with whole cell

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

S.dysenteriae type I. Inherently this bacteria would comprise shiga toxin. The antibodies were primarily of the IgM type, with a low background of IgG (see figure 4). Therefore, the reference teaches the claimed special technical feature of claim 40.

Claims 1-21 and 30-39 lack an inventive step under PCT Article 33(3) as being obvious over Konadu in view of Lees. Konadu teaches the formulation of Escherichia coli O157:H7-shiga toxin conjugates and shows the use of hydrazinolysis and acetic acid hydrolysis in the production of the linked conjugates but differs from the instantly claimed invention by failing to show the use of the recited linker. Lees et al suggest the production of protein-polysaccharide conjugates which would comprise E.coli O-specific polysaccharide(chart, column 11) and show the use of 1-cyano-4-(N,N-dimethoylamino)pyridinium tetrafluoroborate in the production of the conjugates. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the composition of Konadu with the linker of Lees because Lees teaches that the linker enhances the immunogenic characteristics of carbohydrate containing antigens (col. 1, lines 28) and is a conjugation process that is gentle, maintains the integrity of the structure of the carbohydrate and proteins, preserves epitopes in the compounds, is easy to perform, reliable, readily reproducible, is readily scaled up and works with a wide variety of polysaccharide (col. 4, lines 56-61). The person of ordinary skill in the art would have been motivated by the reasonable expectation of success of obtaining conjugates that are useful in the induction and production of an immune response against Escherichia coli O157:H7 a known virulent pathogen.

Claims 27-29 and 41 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998). Konadu suggests the production of antibodies for the administration of patients for treatment of E.coli O157:H7 infection during an outbreak, wherein the antibodies would be produced through administration of the polysaccharide conjugate to a host to induce high tittered IgG anti-lipopolysaccharide globulin. The reference showed the production of antibodies to both the polysaccharide and to Shiga toxin, wherein the shiga toxin antibodies had antigen neutralizing activity. Therefore, the person of ordinary skill in the art at the time the invention was made would have been motivated by the reasonable expectation of obtaining antibodies directed against O157 specific polysaccharide to provide a means of treatment of a patient in a method of passive immunization because Konadu teaches that through the use of antibiotic treatment, the incidence of HUS is potentially increased through the lysis and release of addition shiga toxins. Therefore, administration of antibody compositions would aid in treatment and avoidance of complications that aggravate the disease condition of the patient and serum IgG antibodies directed against E.coli O157:H7 have been successfully produced and antibodies directed against shiga toxin with neutralizing activity have also been obtained through the use of immunogens that comprise both polysaccharide and carrier protein components.

Claims 1-3 and 36 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998) in view of Cryz et al (1990). See discussion of Konadu above. The reference teaches the production of polysaccharide-protein conjugates that comprise E.coli O157specific polysaccharide linked to Shiga toxin but differs from the instantly claimed invention by failing to show the linker to be adipic acid dihydrazide. Cryz et al show the use of adipic acid dihydrazide in the formulation of E.coli o-specific polysaccharide-protein conjugate vaccines in an analogous art for the purpose of producing nontoxic vaccine compositions that elicit a protective immune response. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the composition of Konadu with the linker of Cryz because Cryz teaches that through the use of adipic acid dihydrazide as the linker nontoxic, immunogenic vaccines that comprise both a polysaccharide and a protein component can be combined to elicit a protective immune response directed against E.coli.

Claims 10-11 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998) in view of any one of Porro, Penny or Jennings or Marburg. See discussion of Konadu above. The reference teaches the production of polysaccharide-protein conjugates that comprise E.coli O157 specific polysaccharide linked to Shiga toxin but differs from the instantly claimed invention by failing to show the linker used. Porro, Penny or Jennings or Marburg all show the use of linkers in the formulation o-specific polysaccharide-protein conjugate vaccines in an analogous art for the purpose of producing nontoxic vaccine compositions that elicit a protective immune response and are particularly suitable for immunization of human infants against infection. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the composition of Konadu with the linker of Porro, Penny, Jennings or Marburg because all these references teach that through the use of a linker nontoxic, immunogenic vaccines that comprise both a polysaccharide and a protein component can be combined to elicit a protective immune response that is directed against E.coli.

Claims 1,10-11, 13-17 and 34-39 lack an inventive step under PCT Article 33(3) as being obvious over Robbins in view of Sjogren et al (1987) and Mond. Robbins et al suggest the use of the B subunit of Shiga toxin as a carrier protein in the production of O-specific polysaccharide-protein conjugate compositions and teach that some E.coli strains express shiga toxin when it has been transferred. The reference differs from the instantly claimed invention by failing to show that E.coli O157:H7 expresses shiga toxin. Sjogren et al teach that E.coli O157 and O26 both express Shiga toxin like proteins in an

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

analgous art for the purpose of showing the virulence factors associated with diaherrial disease. Mond claims conjugates of a bacterial polysaccharide with a protein carrier for the realized advantage provided through the combination of both a T-cell independent antigen and a T-cell dependent antigen to produce a protective immune response. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made, to modify the composition of Robbins with the O-specific polysaccharide of Sjogren because both Shigella and E.coli O157 express shiga toxins and the production of vaccine compositions which comprise both a polysaccharide and a protein component have been shown to induce antibodies that are protective.

Claims 34-36 and 39 lack an inventive step under PCT Article 33(3) as being obvious over Robbins in view of Gupta or Taylor. Robbins suggests the use of Shiga toxin beta subunit in the formulation of polysaccharide-protein conjugates with polysaccharide derived from Shigalla species and teach that these compositions are useful in the stimulation of an immune response against enteric pathogens but differs from the instantly claimed invention by failing to show the use of E.coli O111 o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate. Gupta et al show the use of O111-o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate in an analogous art for the purpose of inducing a protective immune response against E.coli strains that cause infantile diarrhea. Taylor shows the use of Shigella dysenteriae O-specific polysaccharide in the production of o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate for the purpose of inducing a protective immune response. Therefore, the references suggest and teach the claimed special technical feature of using Shiga toxin B subunit as a carrier protein in association with a polysaccharide and the recited polysaccharide have been shown previous be useful in the formulation of o-specific polysaccharide in the formulation polysaccharide-protein conjugates to induce an immune response in a host.

---------- NEW CITATIONS ----------
NONE

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	
International application No. PCT/US98/14976	**Applicant's or agent's file reference** 2026-4282PC
International filing date (day/month/year) 20 July 1998 (20.07.98)	**Priority date** (day/month/year)
Applicant SZU, Shousun, C. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 February 2000 (04.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

US9814976

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 2026-4282PC

Box No. I TITLE OF INVENTION

VACCINE AGAINST ESCHERICHIA COLI O157 INFECTION

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

The Government of the United States of America
as represented by the Secretary, Department of
Health and Human Services
Office of Technology Transfer
National Institutes of Health
6011 Executive Boulevard, Suite 325
Rockville, Maryland 20852
US

☐ This person is also inventor.

Telephone No. (301) 496-7056

Facsimile No. (301) 758-6849

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SZU, Shousun C.
9402 Wildoak Drive
Bethesda, Maryland 20814
US

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

FEILER, William S. and MORRY, Mary J.
Morgan & Finnegan, L.L.P.
345 Park Avenue
New York, New York 10154
US

Telephone No. (212) 758-4800

Facsimile No. (212) 751-6849

Teleprinter No. 421792

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

KONADU, Edward
Building 6, Rm 1A06
National Institutes of Health
Bethesda, Maryland 20892
US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ROBBINS, John B.
3901 Rosemary Street
Chevy Chase, Maryland 20815
US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ **AL** Albania .....
- ☒ **AM** Armenia .....
- ☒ **AT** Austria .....
- ☒ **AU** Australia .....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina .....
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria .....
- ☒ **BR** Brazil .....
- ☒ **BY** Belarus .....
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China .....
- ☒ **CU** Cuba .....
- ☒ **CZ** Czech Republic .....
- ☒ **DE** Germany .....
- ☒ **DK** Denmark .....
- ☒ **EE** Estonia .....
- ☒ **ES** Spain .....
- ☒ **FI** Finland .....
- ☒ **GB** United Kingdom
- ☒ **GE** Georgia .....
- ☒ **GH** Ghana .....
- ☒ **GM** Gambia
- ☒ **GW** Guinea-Bissau .....
- ☒ **HR** Croatia
- ☒ **HU** Hungary .....
- ☒ **ID** Indonesia
- ☒ **IL** Israel .....
- ☒ **IS** Iceland
- ☒ **JP** Japan .....
- ☒ **KE** Kenya .....
- ☒ **KG** Kyrgyzstan .....
- ☒ **KP** Democratic People's Republic of Korea .....
- ☒ **KR** Republic of Korea .....
- ☒ **KZ** Kazakhstan .....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho .....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova .....
- ☒ **MG** Madagascar .....
- ☒ **MK** The former Yugoslav Republic of Macedonia .....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi .....
- ☒ **MX** Mexico .....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand .....
- ☒ **PL** Poland .....
- ☒ **PT** Portugal .....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation .....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia .....
- ☒ **SK** Slovakia .....
- ☒ **SL** Sierra Leone
- ☒ **TJ** Tajikistan .....
- ☒ **TM** Turkmenistan .....
- ☒ **TR** Turkey .....
- ☒ **TT** Trinidad and Tobago .....
- ☒ **UA** Ukraine .....
- ☒ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☒ **UZ** Uzbekistan .....
- ☒ **VN** Viet Nam .....
- ☒ **YU** Yugoslavia .....
- ☒ **ZW** Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	4
description (excluding sequence listing part)	:	32
claims	:	5
abstract	:	1
drawings	:	0
sequence listing part of description	:	0
Total number of sheets	:	42

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney (unsigned)
3. ☒ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Mary J. Morry
Agent for Applicants

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: 2026-4282PC

Applicant: The Government of the United States of America as represented by the Secretary, Department of Health and Human Services, et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . $ 240.00 [T]

2. SEARCH FEE . . . . . $ 700.00 [S]

International search to be carried out by US

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 42 sheets.

first 30 sheets . . . . . $ 455.00 [b1]

12 (remaining sheets) x $10.00 (additional amount) = $ 120.00 [b2]

Add amounts entered at b1 and b2 and enter total at B . . . . $ 575.00 [B]

Designation Fees

The international application contains 76 designations.

11 (number of designation fees payable *(maximum 11)*) x $105.00 (amount of designation fee) = $1,155.00 [D]

Add amounts entered at B and D and enter total at I . . . . . $1,730.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . [P]

5. TOTAL FEES PAYABLE . . . . . $2,670.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

[ ] The designation fees are not paid at this time.

MODE OF PAYMENT

[X] authorization to charge deposit account (see below)
[ ] bank draft
[ ] coupons
[ ] cheque
[ ] cash
[ ] other *(specify):*
[ ] postal money order
[ ] revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ US

[X] is hereby authorized to charge the total fees indicated above to my deposit account.

[X] is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

[X] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

THIS SHEET IS FILED IN TRIPLICATE.

Deposit Account No.	Date *(day/month/year)*	Signature
13-4500	20 July 1998	Mary Morry Mary J. Morry

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

2026-4282

To: WILLIAMS S. FEILER
MORGAN AND FINNEGAN, L.L.P.
345 PARK AVENUE
NEW YORK, NEW YORK 10154

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	20 JUL 2000
Applicant's or agent's file reference: 2026-4282PC	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/14976	20 JULY 1998	NONE

International Patent Classification (IPC) or both national classification and IPC
IPC(7): A61K 39/385 and US Cl.: 424/193.1, 196.11, 197.11

Applicant
THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I [X] Basis of the opinion
- II [ ] Priority
- III [ ] Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
- IV [X] Lack of unity of invention
- V [X] Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [ ] Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 20 NOVEMBER 2000.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	GINNY PORTNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (July 1998)*

DOCKETED for 9/20/00

7/25/00

WRITTEN OPINION

International application No.
PCT/US98/14976

I. Basis of the opinion

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-32 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the claims:
pages 33-37 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

WRITTEN OPINION

International application No.
PCT/US98/14976

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees the applicant has:

- [ ] restricted the claims. (See Supplemental Sheet)
- [X] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1 not to invite the applicant to restrict or pay additional fees:

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

- [X] all parts.
- [ ] the parts relating to claims Nos. _.

WRITTEN OPINION

International application No.
PCT/US98/14976

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	2-5,7,9,27-29,35-39,41	YES
	Claims	1,6,8,10-26,30-34,40	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-41	NO
Industrial Applicability (IA)	Claims	1-41	YES
	Claims	NONE	NO

2. citations and explanations

Claims 1,6,8, 10-17, 19-26, 30-34 and 40 lack novelty under PCT Article 33(2) as being anticipated by Konadu et al (1998).

Konadu et al disclose Escherichia coli O157:H7, O specific polysaccharide-B subunit of Shiga toxin 1(see page 386, col. 2, first paragraph). The antibodies induced were neutralizing antibodies directed against the Shiga toxin. The reference suggests that evaluation of other Shiga toxin toxoid proteins as carriers. Administration of the disclosed composition to mice would be in a pharmaceutical carrier and therefore inherently comprises a pharmaceutical carrier with the polysaccharide-protein conjugate composition. The use of recombinant Pseudomonas aeruginosa exoprotein A as a carrier protein is also disclose (title of article). The dose for the administered polysaccharide is disclosed to be 25 ug of the E.coli O157:H7 polysaccharide (page 384, col. 1, paragraph 1). A method of inducing an immune response using the disclosed vaccine composition is disclosed to have induced antibodies to both the polysaccharide and the shiga toxin carrier protein. Clinical trials in humans were shown to provide encouraging test results, wherein one human subject upon infection with E.coli O157:H7 after having been vaccinated with the conjugate composition evidenced a positive stool culture for Ecoli O157:H7 but not adverse reaction and a negative stool culture at repeat testing (page 384, col. 1, clinical response). Serum samples obtained from patients evidenced immunoreactivity against Shiga toxin 1 beta subunit and therefore anticipates the claimed antibody compositions of claim 40.

Claims 10-22 and 24-26 lack novelty under PCT Article 33(2) as being anticipated by Konadu et al (1994).

Konadu et al disclose the production of Escherichia coli O157:H7 polysaccharide-protein conjugates for use as vaccines, wherein the conjugates are produced with a hydrazine linker or through acetic acid hydrolysis. Antibodies specific to both the polysaccharide and the protein carrier where identified in (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

IV. LACK OF UNITY OF INVENTION:

1. This response is made to a telephone Lack of Unity requirement (see telephone memorandum attached hereto or attached to a prior Written Opinion).

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
serum samples taken after vaccination of the host. Therefore, the reference anticipates the now claimed invention.

Claim 40 lacks novelty under PCT Article 33(2) as being anticipated by Chu et al(1991).

Chu et al disclose a composition of antibodies produced through the immunization of a host animal with whole cell S.dysenteriae type I. Inherently this bacteria would comprise shiga toxin. The antibodies were primarily of the IgM type, with a low background of IgG (see figure 4). Therefore, the reference teaches the claimed special technical feature of claim 40.

Claims 1-21 and 30-39 lack an inventive step under PCT Article 33(3) as being obvious over Konadu in view of Lees. Konadu teaches the formulation of Escherichia coli O157:H7-shiga toxin conjugates and shows the use of hydrazinolysis and acetic acid hydrolysis in the production of the linked conjugates but differs from the instantly claimed invention by failing to show the use of the recited linker. Lees et al suggest the production of protein-polysaccharide conjugates which would comprise E.coli O-specific polysaccharide(chart, column 11) and show the use of 1-cyano-4-(N,N-dimethoylamino)pyridinium tetrafluoroborate in the production of the conjugates. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the composition of Konadu with the linker of Lees because Lees teaches that the linker enhances the immunogenic characteristics of carbohydrate containing antigens (col. 1, lines 28) and is a conjugation process that is gentle, maintains the integrity of the structure of the carbohydrate and proteins, preserves epitopes in the compounds, is easy to perform, reliable, readily reproducible, is readily scaled up and works with a wide variety of polysaccharide (col. 4, lines 56-61). The person of ordinary skill in the art would have been motivated by the reasonable expectation of success of obtaining conjugates that are useful in the induction and production of an immune response against Escherichia coli O157:H7 a known virulent pathogen.

Claims 27-29 and 41 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998). Konadu suggests the production of antibodies for the administration of patients for treatment of E.coli O157:H7 infection during an outbreak, wherein the antibodies would be produced through administration of the polysaccharide conjugate to a host to induce high tittered IgG anti-lipopolysaccharide globulin. The reference showed the production of antibodies to both the polysaccharide and to Shiga toxin, wherein the shiga toxin antibodies had antigen neutralizing activity. Therefore, the person of ordinary skill in the art at the time the invention was made would have been motivated by the reasonable expectation of obtaining antibodies directed against O157 specific polysaccharide to provide a means of treatment of a patient in a method of passive immunization because Konadu teaches that through the use of antibiotic treatment, the incidence of HUS is potentially increased through the lysis and release of addition shiga toxins. Therefore, administration of antibody compositions would aid in treatment and avoidance of complications that aggravate the disease condition of the patient and serum IgG antibodies directed against E.coli O157:H7 have been successfully produced and antibodies directed against shiga toxin with neutralizing activity have also been obtained through the use of immunogens that comprise both polysaccharide and carrier protein components.

Claims 1-3 and 36 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998) in view of Cryz et al (1990). See discussion of Konadu above. The reference teaches the production of polysaccharide-protein conjugates that comprise E.coli O157specific polysaccharide linked to Shiga toxin but differs from the instantly claimed invention by failing to show the linker to be adipic acid dihydrazide. Cryz et al show the use of adipic acid dihydrazide in the formulation of E.coli o-specific polysaccharide-protein conjugate vaccines in an analogous art for the purpose of producing nontoxic vaccine compositions that elicit a protective immune response. Therefore, it would have been obvious to the person of ordinary skill

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII Sheet 11

in the art at the time the invention was made to modify the composition of Konadu with the linker of Cryz because Cryz teaches that through the use of adipic acid dihydrazide as the linker nontoxic, immunogenic vaccines that comprise both a polysaccharide and a protein component can be combined to elicit a protective immune response directed against E.coli.

Claims 10-11 lack an inventive step under PCT Article 33(3) as being obvious over Konadu (1998) in view of any one of Porro, Penny or Jennings or Marburg. See discussion of Konadu above. The reference teaches the production of polysaccharide-protein conjugates that comprise E.coli O157 specific polysaccharide linked to Shiga toxin but differs from the instantly claimed invention by failing to show the linker used. Porro, Penny or Jennings or Marburg all show the use of linkers in the formulation o-specific polysaccharide-protein conjugate vaccines in an analogous art for the purpose of producing nontoxic vaccine compositions that elicit a protective immune response and are particularly suitable for immunization of human infants against infection. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made to modify the composition of Konadu with the linker of Porro, Penny, Jennings or Marburg because all these references teach that through the use of a linker nontoxic, immunogenic vaccines that comprise both a polysaccharide and a protein component can be combined to elicit a protective immune response that is directed against E.coli.

Claims 1,10-11, 13-17 and 34-39 lack an inventive step under PCT Article 33(3) as being obvious over Robbins in view of Sjogren et al (1987) and Mond. Robbins et al suggest the use of the B subunit of Shiga toxin as a carrier protein in the production of O-specific polysaccharide-protein conjugate compositions and teach that some E.coli strains express shiga toxin when it has been transferred. The reference differs from the instantly claimed invention by failing to show that E.coli O157:H7 expresses shiga toxin. Sjogren et al teach that E.coli O157 and O26 both express Shiga toxin like proteins in an analgous art for the purpose of showing the virulence factors associated with diaherrial disease. Mond claims conjugates of a bacterial polysaccharide with a protein carrier for the realized advantage provided through the combination of both a T-cell independent antigen and a T-cell dependent antigen to produce a protective immune response. Therefore, it would have been obvious to the person of ordinary skill in the art at the time the invention was made, to modify the composition of Robbins with the O-specific polysaccharide of Sjogren because both Shigella and E.coli O157 express shiga toxins and the production of vaccine compositions which comprise both a polysaccharide and a protein component have been shown to induce antibodies that are protective.

Claims 34-36 and 39 lack an inventive step under PCT Article 33(3) as being obvious over Robbins in view of Gupta or Taylor. Robbins suggests the use of Shiga toxin beta subunit in the formulation of polysaccharide-protein conjugates with polysaccharide derived from Shigalla species and teach that these compositions are useful in the stimulation of an immune response against enteric pathogens but differs from the instantly claimed invention by failing to show the use of E.coli O111 o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate. Gupta et al show the use of O111-o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate in an analogous art for the purpose of inducing a protective immune response against E.coli strains that cause infantile diarrhea. Taylor shows the use of Shigella dysenteriae O-specific polysaccharide in the production of o-specific polysaccharide in the formulation of a polysaccharide-protein conjugate for the purpose of inducing a protective immune response. Therefore, the references suggest and teach the claimed special technical feature of using Shiga toxin B subunit as a carrier protein in association with a polysaccharide and the recited polysaccharide have been shown previous be useful in the formulation of o-specific polysaccharide in the formulation polysaccharide-protein conjugates to induce an immune response in a host.

---------- NEW CITATIONS ----------
NONE

ATTACHMENT TO CHAPTER II PCT TELEPHONE MEMORANDUM FOR LACK OF UNITY OF INVENTION

Itemized Summary Of Claim Groupings:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)S 1-9, 19-21 and 30-33, drawn to E.coli O157 O-specific polysaccharide conjugates which are covalently bound to one of four(4) different protein carriers, wherein the carrier is derived from Shiga toxin 1 or 2.

Group II, claim(s)10-18, drawn to E.coli O157 polysaccharide covalently bound to any protein, wherein various at least 6 species of protein carriers are recited.

Group III, claim(s) 22-26, drawn to antibodies which are immunoreactive with E. coli O157 O-specific polysaccharide.

Group IV, claim(s)27-29, drawn to a method of passively immunizing a host against O157 infection.

Group V, claim(s) 34-39, drawn to conjugates comprising O-specific polysaccharide from E.coli or Shigella dysentariae,(at least 4 different sources are recited) together with any one of four different protein carriers.

Group VI, claim(s)40, drawn to antibodies which are immunoreactive with Shiga toxin 1 or 2.

Group VII, claim(s) 41, drawn to a method of administering antibodies to a mammal.

ATTACHMENT TO CHAPTER II PCT TELEPHONE MEMORANDUM FOR LACK OF UNITY OF INVENTION

Detailed Reasons For Holding Lack Of Unity Of Invention:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

GROUP I:(1) O157-BETA SUBUNIT OF SHIGA TOXIN 1, (2) O157-BETA SUBUNIT OF SHIGA TOXIN 2, (3)O157-NON-TOXIC MUTANT SHIGA TOXIN 1, HOLOTOXIN, (4)O157-NON-TOXIC MUTANT SHIGA TOXIN 2, HOLOTOXIN. GROUP II: (1)O157-TOXOID CONJUGATE, (2) O157-CLOSTRIDIUM TOXOID OR EXOTOXIN, (3)O157-PSEUDOMONAS AERUGINOSA RECOMBINANT EXOPROTEIN A, (4)O157-HEPATITIS B SURFACE ANTIGEN, (5)O157-HEPATITIS B CORE ANTIGEN, (6)O157-BOVINE SERUM ALBUMIN. GROUP V:(1)O111-SHIGA TOXIN, (2)O17-SHIGA TOXIN, (3)O26-SHIGA TOXIN, (4) SHIGELLA DYSENTERIAE O-SPECIFIC POLYSACCHARIDE-SHIGA TOXIN.

The inventions listed as Groups I,II,III,IV,V,VI,and VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: each of the inventions differ in the structural components used in the invention and therefore differ in the function and effect derived from each, as well as the special technical feature set forth in Group II is known in the art, specifically O-specific polysaccharide-protein conjugates of Escherichia coli O157 to bovine serum albumin, Clostridium welchii exotoxin and Pseudomonas aeruginosa recombinant exoprotein A and therefore does not define an advancement in the art; therefore a special technical feature is not set forth therein.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each of the species contained in the different Groups comprise structural proteins or O-specific polysaccharide which are associated with differing diseases and contain different types of amino acids or sugars which in turn define differing structural components which work to produce different functions and effects. Therefore, each specifies defines a different invention.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

1999 JUN 11 P 2:23

FEILER, William, S.
Morgan & Finnegan, L.L.P.
345 Park Avenue
New York, NY 10154
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 04 June 1999 (04.06.99)	
Applicant's or agent's file reference 2026-4282PC	**IMPORTANT NOTIFICATION**
International application No. PCT/US98/14976	**International filing date** (day/month/year) 20 July 1998 (20.07.98)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
KONADU, Edward	GH	GH
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
KONADU, Yvonne Ageyman House No. Plot 3, 2nd Street Asokore Mampong Ashanti Region Ghana	GH	GH
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
KONADU, Edward has been recorded as deceased inventor. The person in box 2, heiress of the deceased inventor, has been recorded as applicant for the US.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Maria Victoria CORTIELLO
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

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2026-4282 PC MJM

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: WILLIAMS S. FEILER
MORGAN AND FINNEGAN, L.L.P.
345 PARK AVENUE
NEW YORK, NEW YORK 10154

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	28 NOV 2000
Applicant's or agent's file reference: 2026-4282PC	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US98/14976	20 JULY 1998	NONE

Applicant

THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer GINNY PORTNER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*